PRODUCTION OF GLUCOSE FROM BANANA STEM WASTE USING *STRAIN A*

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ABSTRACT

Fermentable sugars are the largest feedstock available to support bio-based chemicals industry. Growth of a bio-based chemicals industry will depend on production of fermentable glucose. Production of this glucose from banana stem waste can help to reduce the environmental problem. This study is studying glucose production from banana stem waste using *Strain A*. While, the purposes of this study are to study the effect of organic loading rate (OLR) during production of glucose, to optimize the glucose production using banana stem waste and to study suitability of banana stem waste as substrate for glucose production using *Strain A*. Organic loading rate for this study is determined by using Design Expert. The banana stem waste is used as substrate and contains 27.64% of total solid. The experiment used fed batch fermentation. The fermented mixture were removed 50 mL and after that, 50 mL of fresh substrate is added back to shake flask. This experiment be done for eight runs with different values of OLR (g L^{-1} d^{-1}); 5, 11.25, 17.5, 23.75, and 30. The experiment was also carried out for 30 days. The concentration of glucose is analyzed using DNS assay. The optimization of glucose production is determined using Design Expert through One Factor Analysis. The increasing yield of glucose is affected by decreasing the value of OLR. The optimum OLR is 5 g L^{-1} d^{-1} was produced the maximum of yield of glucose and it is 0.0734 g/g substrate. The banana stem waste is suitable substrate in production of glucose using biological hydrolysis process and *Strain A*.

ABSTRAK

Glukosa yang diperolehi daripada fermentasi adalah bahan utama yang tersedia untuk menyokong industri bahan kimia berasaskan bio. Pertumbuhan industri bahan kimia berasaskan bio bergantung pada penghasilan glukosa. Penghasilan glukosa ini dari sisa batang pisang dapat membantu mengurangkan masalah pencemaran. Kajian ini mengkaji penghasilan glukosa dari sisa batang pisang menggunakan *Strain A*. Manakala, tujuan kajian ini adalah untuk mengkaji kesan kadar beban organik (OLR) terhadap penghasilan glukosa, mengoptimumkan penghasilan glukosa menggunakan sisa batang pisang dan mengkaji kesesuaian sisa batang pisang sebagai substrat untuk penghasilan glukosa menggunakan *Strain A*. Kadar beban organik untuk kajian ini ditentukan dengan menggunakan Design Expert. Sisa batang pisang yang digunakan sebagai substrat mengandungi 27.64% dari jumlah keseluruhan pepejal. Eksperimen ini mengunakan teknik penapaian secara suapan berkelompok. Substrat yang telah ditapai dikeluarkan sebanyak 50 mL dan selepas itu, 50 mL substrat yang baru akan dimasukkan kembali ke dalam tempat penapaian. Penapaian ini dijalankan sebanyak lapan kali dengan nilai kadar beban organik (OLR) yang berbeza-beza iaitu 5 g L^{-1} h^{-1}, 11.25 g L^{-1} h^{-1}, 17.5 g L^{-1} h^{-1}, 23.75 g L^{-1} h^{-1}, dan 30 g L^{-1} h^{-1}. Kajian ini juga dijalankan selama 30 hari. Kepekatan glukosa dianalisa menggunakan kaedah DNS. Penghasilan glukosa yang optimum ditentukan dengan menggunakan Design Expert melalui Analisis Satu Faktor. Peningkatan dalam penghasilan glukosa dipengaruhi oleh penurunan nilai OLR. Nilai OLR yang optimum adalah 5 g L^{-1} h^{-1} telah menghasilkan maksimum glukosa dan nilainya adalah 0.0734 g / g substrat. Sisa batang pisang adalah substrat yang sesuai dalam penghasilan glukosa melalui proses hidrolisis secara biologi dan *Strain A*.

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LIST OF ABBREVIATIONS/ SYMBOLS

[glucose]	-	Concentration of glucose
[substrate]	-	Concentration of substrate
˚ C	-	Degree Celsius
CSTR	-	Continuous Stirrer Reactor
d	-	Day
DNS	-	Dinitrosalicyclic Acid Reagent
DP	-	Degree of Polymerization
g	-	Gram
h	-	Hari
hr	-	Hour
HRT	-	Hydraulic retention time
L	-	Liter
mL	-	Milliliter
nm	-	Nanometer
OD	-	Optical density
OLR	-	Organic loading rate
TS	-	Total solid
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background

Glucose is an organic compound and known as monosaccharide (a simple sugar). It is also known as grape sugar, blood sugar, or corn sugar. Its molecular formula is $C_6H_{12}O_6$ and contains aldehyde group. When glucose units in long chains form, it is called polysaccharides. The types of polysaccharides are cellulose, glycogen and starch. Glucose is commonly available in the form of a white substance or as a solid crystal. It can also be dissolved in water as an aqueous solution.

Glucose is produced commercially via the hydrolysis, enzymatic hydrolysis of starch or biomass waste (Moshe and Reese, 1968) and fermentation. The sources of starch are sugarcane bagasse (Martin *et al*., 2007), rice (Yanez *et al*., 2006), wheat, cassava, corn husk (Ohgren *et al.*, 2007), sago and all source of organic. Agriculture waste also widely used in the production of glucose as alternative sources.

In nature, many microorganisms such fungus or bacteria can degrade the cellulosic materials. Microorganisms (single culture) that used to degrade the cellulosic material have been well studied (Hayashi *et al.*, 1999, Del Re *et al.*, 2003, Lo *et al.*, 2008). Mixed cultures also have been well studied to degrade the cellulose (Lewis *et al.*, 1988). The process to degrade the cellulose by microorganisms is called bacterial hydrolysis or biological hydrolysis. This hydrolysis could degrade the cellulosic materials aerobically and anaerobically (Lo *et al.*, 2008).

Fermentation is the process formed of energy by the process of oxidation of organic compounds like carbohydrates and sugars. Anaerobic fermentation is fermentation that carried out without the presence of oxygen. While, when the fermentation is carried out with oxygen, it is commonly known as aerobic fermentation. Each microorganism has its condition of fermentation. For example, when use microbe from soil, the anaerobic fermentation must be applied because the soil fungi are facultative anaerobic organisms able to change their energy metabolisms depending on aeration conditions (Tetsubin *et al.*, 2003).

1.2 Problem Statement

Nowadays, many people and industries were aware about environmental problem. The agriculture waste or biomass is one of the problems to environmental. The amount of agriculture waste was increases by days and causes the serious problem to environment. With increasing environmental awareness, the conversion of biomass and agriculture waste into chemical is receiving an increased interest.

Biomass and agriculture waste can convert into any bio- product, but before that, the waste need convert to glucose. Fermentable sugars are the largest feedstock available to support bio-based chemicals industry. Growth of a bio-based chemicals

industry will depend on production of fermentable glucose. Fermentable glucose is also used in foods, medicine, brewing, and wine making and as the source of various other organic chemicals.

Production of this glucose from banana stem waste is the good chosen. It is because banana stem waste is cheap (low cost) and easy to find in Malaysia. Banana is covering about 26,000 hectares with a total production is 530,000 metric tones in Malaysia. Banana is a most popular fruit and has received demand for food industries. But, banana stem from banana tree will be the waste and became the environmental problem.

1.3 Objective of The Study

The main objectives of this research are:-

1. To study the effect of Organic Loading Rate (OLR) during production of glucose.
2. To optimize the glucose production using banana stem waste.
3. To study suitability of banana stem waste as substrate for glucose production using *Strain A*.

1.4 Scopes of Study

In order to achieve the objectives, the equation of organic loading rate has been identified. The equation of OLR is related to concentration of substrate (banana stem waste) and hydraulic retention time (HRT). The equation of HRT contains

volume of reactor and flow rate. The type of reactor that use in this study is sequencing batch reactor (fed-batch reactor). The value of volume of reactor is 5 liters will used and flow rate will fix, so that HRT will be fixed. The value of OLR is varied. Then, the value of concentration of substrate will get with using equation OLR and the other method is using the Design Expert. Total solid (TS) test will used to make sure the substrate has similar moisture for each run of experiment. The other scope is type of substrate. The banana stem waste will use as substrate in this study. The method to measure concentration of glucose in this study is DNS assay. Design Expert also used to optimize the glucose production using method One Factor Analysis. The suitability of banana stem waste as substrate is determined from glucose that produced and compared it with substrate that used from previous study.

CHAPTER 2

LITERATURE REVIEW

2.1 Fermentable Glucose/ Fermentable Sugar

2.1.1 Properties of Fermentable Glucose

Fermentable glucose is known as glucose or simple sugar. Glucose is an example of a carbohydrate. Molecular formula and molar mass of glucose is $C_6H_{12}O_6$ and 180.16 g/mol. Figure 2.1 shows the structure of glucose that contains six carbons and aldehyde group.

According Forest Encyclopedia (2008), fermentable sugars can be produced using crops and wastes from agriculture and forestry. The types of crop that always used to produce fermentable sugar are corn, wheat, potato, sugar beet, and sugarcane. Besides that, potato-processing residues, cane molasses, and apple pomace (Polman 1994).

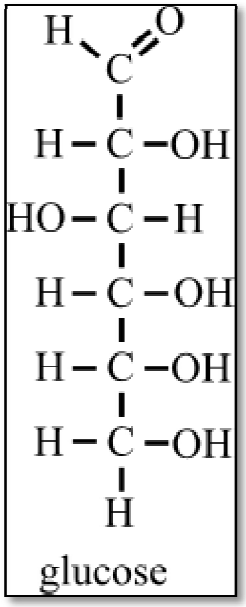

Figure 2.1: The Structure of glucose.

Sugars can be produced directly or derived from polysaccharides such as cellulose and starch and then, via microbial fermentation to produce a wide range of others chemicals. Glucose is produced commercially via the enzymatic hydrolysis of starch. Besides that, cellulose can be hydrolyzed by acid to glucose, although much of the glucose is destroyed during this process.

2.1.2 Application of Fermentable Glucose

Glucose is used as a precursor for the synthesis of several important substances. According Wikipedia, glucose is a precursor for vitamin C (ascorbic acid) production in plants and most animals. In the industry, glucose is also used as a precursor to make vitamin C in the Reichstein process, to make citric acid, gluconic acid, bio-ethanol, polylactic acid and sorbitol.

Besides that, fermentable glucose is used to produce bio-chemical product. It is proven by Forest Encyclopedia (2008) that stated fermentable sugars are the largest feedstock available to support a bio-based chemicals industry. Existing commercial fermentation primarily utilizes glucose to produce ethanol, acetic acid, amino acids, antibiotics, and other chemicals.

2.2 Source of Substrate

Fermentable glucose can be derived by fermentation process from any material that contains celluloses and hemicelluloses. The many and varied raw materials used in the manufacture of fermentable glucose via hydrolysis and fermentation. The most substrate that used to produce fermentable glucose is starch, food waste and agriculture waste.

2.2.1 Starch

Starches are complex sugars that can break down into one of the simple sugars (maltose). Since starches do not taste very sweet, they do not jump to mind when sugar is mentioned, but they quickly become the simple sugar maltose, and then the simple sugar glucose because the breakdown of starch from the complex sugar form to the simple sugar form is quick and easy. Essentially, starches are sugars that merely require a few more steps to make them into glucose.

Figure 2.2 was shown the structure of starch that made up of repeated structure of glucose.

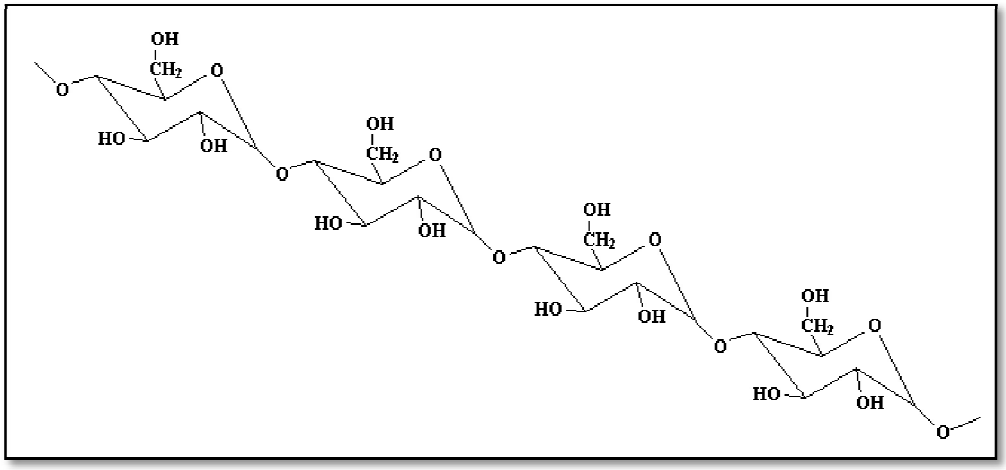

Figure 2.2: The structure of starch.

Starch is found in potatoes, and in grains such as corn and wheat. Besides that, many crops can be used as the source of starch. The example crops that can be used as the source of starch are maize, rice, wheat, potato, cassava, arrowroot, and sago. In the United States, corn starch (from maize) is used almost exclusively. But in Malaysia, cassava (tapioca) starch and sago starch is always used as substrate to produce fermentable glucose and bio-chemical.

Starch also used as source of food to human and animal. Besides that, starch is used in pressing clothes to keep them from wrinkling and to make a foam packing.

2.2.2 Food Waste

Food waste is a kind of organic waste discharged from households, cafeterias and restaurants, and accounts for a considerable proportion of municipal solid garbage. Figure 2.3 shown seven percent of waste is municipal solid waste. It is still be a problem to environmental.

Many researches were done to study the conversion of food waste. Kim *et al*. (2003) researched about conversion of food waste into lactic acid and reported that the lactic acid concentration in the medium of food waste could reach 80 g/L after 48 hr under the catalysis of a commercial enzyme mixture. In previous works, Kim *et al*. had developed a bioprocess for the lactic acid production from food waste, but the lactic acid concentration was still below 30 g/L when the process did not use any commercial enzyme.

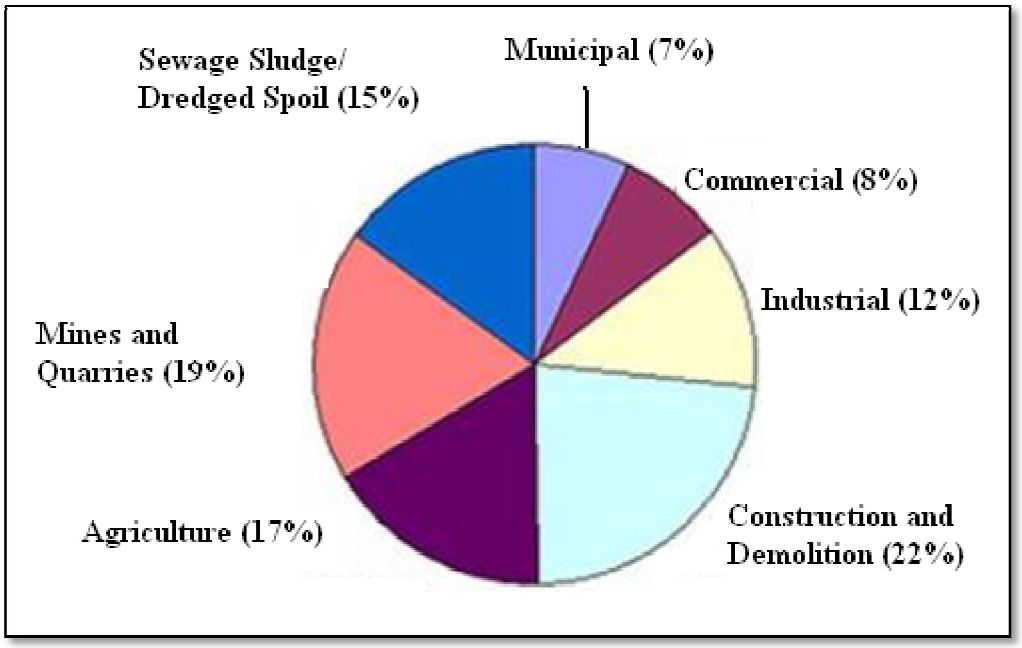

Figure 2.3: Type of waste in global.

2.2.3 Agriculture waste

Agricultural waste is one of the largest segments of the nationwide waste problem. Figure 2.3 was shown the 17% of waste in global is agriculture waste. Agricultural wastes include both natural (organic) and non-natural. The large volumes of agricultural waste threaten surface water and groundwater quality in the event of waste spills, leakage from waste storage facilities, and run off from fields on

which an excessive amount of waste has been applied as fertilizer. Agriculture waste also is an environmental problem issues that need to solve quickly.

The organic in agricultural waste contains cellulose and hemicelluloses that can convert into the fermentable glucose and bio-chemical product. Many researches were being done to study the conversion of agriculture waste into valuable product.

Saccharification of banana agro waste by cellulases of *Trichoderma lignorum* was investigated by Baig *et al*. (2004). Banana is major cash crop of this region generating vast agricultural waste after harvest. The agro waste including dried leaves and psuedostem after harvest was used as substrate for the release of sugars (Baig *et al.*, 2004**).** Banana fruit stalk abundantly available in banana production fields and markets appears to be a favorable substrate as it is cheaply available in the tropical and subtropical countries and has a cellulose content of 23.85% (Krishna, 1996).

2.2.4 Selection of Substrate

Over the long term, new sources of glucose will be required to meet the demands of a bio-based industry. Growth of a bio-based chemicals industry will depend on substrate that contains cellulose. Based on list of substrate, agricultural waste (banana waste) has been chosen as source of substrate in this study. Banana waste was been chosen because it is easy to find in Malaysia. Selection of banana waste is based on to reduce environmental problem. Besides that, it is better not to disturb source of food such as starch.

2.3 Hydrolysis Process

2.3.1 Enzymatic Hydrolysis

Enzymatic hydrolysis is widely use in production of fermentable glucose. This hydrolysis uses enzymes that produced by a variety of microorganisms. Those enzymes must capable to break down lignocellulosic material and convert it to fermentable glucose. The advantage of this hydrolysis is does not having the same problem with the acid hydrolysis that causes corrosion of equipment. Besides that, enzymatic hydrolysis using mixtures of enzymes, such as cellulase and hemicellulaces, is used to avoid the destruction of sugars associated with acid treatments (hydrolysis) of lignocellulosic material. These enzymes, when combined with effective pretreatment of lignocellulosics, provide high yields of glucose, xylose, and other fermentable sugars with minimal sugar losses.

In addition, this hydrolysis has low utility cost compared to acid hydrolysis and low environmental conditions (Sun and Cheng, 2002 and Puwardi, 2006). However, these enzymes are currently too costly to use in large-scale conversion of lignocellulosic materials to fermentation substrates. This hydrolysis also requires longer retention time and the rate of hydrolysis is very slow (Puwardi, 2006 and Kumar *et al*., 2009).

Basically, there are two major processes involve in enzymatic hydrolysis; liquefaction and saccharification. Aggarwal *et al*. (2001) use this hydrolysis to convert starch to glucose. Aggarwal *et al* liquefaction under pressurized steam and found that technique more effective than using water bath.

While, saccharification is refers to production of fermentable sugar from polysaccharides (Dunson *et al*., 2007). The saccharification was improved with the increasing enzymes unit. Effect of addition of divalent ions on the process of

saccharification was studied by the addition of calcium chloride, magnesium and zinc sulphate to provide these ions in the range of 25-250 mg/l. Results obtained for the level of saccharification in presence of these ions (Aggarwal *et al*., 2001).

In many cases relatively high doses of glucoamylases and other maceration enzymes besides amylases such as xylanase, cellulose and pectinase are necessary to saccharify various starches containing substrates efficiency. Moreover, the efficiency of an enzymatic starch saccharification process depends on the activity of the glucoamylase and also on the purity of enzyme (Aggarwal *et al*., 2001).

2.3.2 Acid Hydrolysis

Initially, acid hydrolysis appears to be a relatively efficiently means of accessing and breaking down cellulose. The hydrogen ion, therefore, does not face the problem of accessibility compared to cellulose enzymes.

Initial hydrolysis rates are typically very rapid performed experiments to show that in the initial stages of the hydrolysis reaction, larger pore volumes do correspond to faster reaction rates. However, after limited hydrolysis, the reaction rate slows down considerably. The glycosidic bonds most susceptible to hydrolysis are those either at the surfaces or in the amorphous region of cellulose. Rapid hydrolysis rates reflect hydrolysis activity in these regions and can be seen as a decrease in the degree of polymerization (DP) from several thousand to about 200.

The cellulose can then be rapidly hydrolyzed at low temperature to avoid degradation, making almost quantitative yield of glucose attainable. However, in the process, high capital cost is an avoidable because of expensive corrosion resistant

equipment, acid recovery plants and higher operation costs. Moreover, one of the major problems with hydrolyzates produced by acid hydrolysis is the poor ferment ability caused by the presence of inhibitors in the hydrozylates. Furfural is known to be one of the most important of these inhibitors. It is a breakdown product from pentose and is formed in a browning reaction during hydrolysis in the presence strong acids. It therefore may be impossible to completely avoid furfural formation in a chemical hydrolysis process designed to give a high sugar yield (Taherzadeh *et al*., 1999).

2.3.3 Biological Hydrolysis

Waren (1996) was written in his paper that microorganisms are efficient degraders of starch, chitin, and the polysaccharides in plant cell walls. Biological hydrolysis uses the microorganisms to degrade, so, it different with enzymatic hydrolysis. This hydrolysis is always used in waste treatment. While, Chen *et al*. (2009) use this hydrolysis to degrade the starch so that, it can convert starch to glucose.

Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation (Okano *et al*., 2005). In biological pretreatment processes, microorganisms such as brown-, white-, and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Galbe and Zacchi, 2007).

Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidase and laccase (Lee *et al*., 2007).

These enzymes are regulated by carbon and nitrogen sources. White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials. Hatakka *et al*. (1983) studied the pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in five weeks.

The advantage of this hydrolysis is only requiring low energy to degrade the starch, lignin and hemicelluloses (Kumar *et al*., 2009). This hydrolysis also has mild environmental condition.

Biological hydrolysis is defined as the rate limiting step in anaerobic digestion because this hydrolysis can reduce the impact of rate limiting step, pretreatment systems such as thermal, alkaline, ultrasonic and mechanical disintegration systems (Park *et al*., 2005, and Tiehm *et al*., 2001). Besides that, this hydrolysis have a low rate of hydrolysis but this method involves relatively cheaper maintenance costs and suitable for large scale treatment (Park *et al*., 2005).

2.3.4 Selection of Hydrolysis Process

This study will be use biological hydrolysis. This process has been chosen because it is can reduced the cost and suitable for large scale treatment. Enzymatic hydrolysis will give the high yield of sugar but this process is very expensive. Besides that, biological hydrolysis is efficient to degrade the starch, chitin, and the polysaccharides in plant cell walls. It can reduce the usage of other pretreatment method.

2.4 Bioreactor

Fermentations can be operated in batch, fed-batch or continuous bioreactors.

2.4.1 Batch Bioreactor

In batch bioreactor all components, except gaseous substrates such as oxygen, pH-controlling substances and antifoaming agents, are placed in the reactor in the beginning of the fermentation. During process there is no input nor does output flow. Batch bioreactors have several advantages over continuous flow reactors. The advantage is the fermentation can be stopped between batches, so the production rate is flexible and can be varied if economically desirable.

The other advantages are batch bioreactors are also more flexible, in that one can easily use different compositions in different batches to produce products with different specifications and if the reactants are stirred, a batch bioreactor can often achieve better quality than a plug flow reactor and better productivity than a continuous flow reactor.

Batch bioreactor cannot achieve the steady state condition. It will cause wrong interpretation of the results for the full scale implementations (Ucisik and Henze, 2008).

2.4.2 Continuous Stirrer Bioreactor

Continuous stirrer bioreactor always operated at steady state. The characterization continuous stirrer bioreactor (CSTR) is run at steady state with continuous flow of reactants and products; the feed assumes a uniform composition throughout the reactor, exit stream has the same composition as in the tank.

This reactor commonly used in industry processing. Besides that, this type of reactor is used in the real life fermentation application (Ucisik and Henze, 2008). The advantages of CSTR are easy to clean, low operating (labor) cost, easily adapts to two phase runs and it is good temperature control. The disadvantages of CSTR are poor agitation and lowest conversion per unit volume (Fogler, 2006).

2.4.3 Fed Batch Bioreactor

Bushan and Joshi (2004) use this type of bioreactor for produce baker's yeast. This reactor is very popular in the ethanol production. In fed-batch process, the substrate or medium or product will removed from the reactor for few days. Then, the fresh substrate is added in order to control the reaction rate by its concentration. The remove and added process is based on hydraulic retention time (HRT). There are both input and output flows in a continuous process, but the reaction volume is kept constant. In addition, fed batch is using to enhance the biological hydrolysis (Chen *et al*., 2009).

Fed-batch bioreactors are widely used in industrial applications because they combine the advantages from both batch and continuous processes. Process is at first started as a batch process, but it is exhibited from reaching the steady state by